

Assessment of Testicular Testosterone Production and Leydig Cell Structure

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Advances in two techniques have made the problem of assessing the acute and/or chronic effects of toxic agents on Leydig cell structure and testosterone synthesis and secretion amenable to study. First, *in vitro* testicular perfusion has been perfected to a point where it closely resembles *in situ* testosterone secretion. Second, now it is possible to quantify the proportion of Leydig cell cytoplasm occupied by the cellular organelles which contain steroidogenic enzymes. Herein, we report that inhibition of Leydig cell steroidogenic enzymes is reflected by reduced testosterone secretion by *in vitro* perfused rat and rabbit testes. Moreover, the activity of specific steroidogenic reactions can be monitored by measuring the secretion of reaction substrate(s) and product(s) from *in vitro* perfused testes. Testosterone secretion by *in vitro* perfused testes from five species is highly and positively correlated with the volume density of smooth endoplasmic reticulum in Leydig cell cytoplasm. Exploitation of these findings will allow toxicologists to quantitatively assess the effect of toxicants on Leydig cell testosterone biosynthesis and secretion, to identify the specific steroidogenic enzymes affected, to assess whether the membranous environment of the steroidogenic enzymes is compromised, and perhaps even to predict the deleterious effect of a toxic agent on Leydig cell steroidogenic function from a stereological assessment of Leydig cell ultrastructure.

Introduction

The ultrastructure and steroidogenic function of the Leydig cell have been described thoroughly in several reviews (1-4). These reviews indicate that Leydig cells produce the bulk of testicular testosterone and that the testosterone biosynthetic enzymes are sequestered in Leydig cell mitochondria and smooth endoplasmic reticulum (SER). In the adult, testosterone regulates sexual behavior, accessory sex organ function, epididymal sperm maturation, and spermatogenesis (4). Therefore, toxic agents which inhibit testosterone biosynthesis and/or secretion may have a profound effect on any of the myriad processes required for the timely deposition of viable spermatozoa into the female reproductive tract.

Advances in two techniques have made the problem of assessing the acute and/or chronic effects of toxic agents on Leydig cell structure and on testosterone synthesis and secretion amenable to study. First, *in vitro* testicular perfusion has been perfected to a point where it closely resembles *in situ* testosterone secretion. Also, this method now can be used to measure the activity of steroidogenic reactions sequestered in specific Leydig cell cytoplasmic organelles. Second, stereological procedures allow the quantification of three-dimensional structures by extrapolation from the measurement of two-dimensional cross sections. For example, now it is possible to quantify the proportion of Leydig cell cytoplasm occupied by smooth endoplasmic reticulum, the organelle that contains the enzymes responsible for the conversion of pregnenolone to testosterone.

Herein we describe experiments from our laboratory which demonstrate: (1) that steroidogenesis by *in vitro* perfused and *in situ* testes is similar, (2) that testosterone biosynthesis and secretion by *in vitro* perfused testes are diminished dramatically

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by inhibitors of specific steroidogenic enzymes infused into the spermatic artery, (3) that *in vitro* perfused testes can be used to elucidate the site of inhibition in the testosterone biosynthetic pathways, and (4) that the proportion of certain Leydig cell cytoplasmic organelles is correlated with testosterone biosynthesis and secretion. Exploitation of these findings will allow toxicologists to quantitatively assess the effect of xenobiotics on Leydig cell structure and steroidogenic function.

Materials and Methods

The reader is referred to original sources for detailed descriptions of materials and methods.

In Vitro Perfusion

The perfusion method was described originally by Van Demark and Ewing (5) and Ewing et al. (6). The perfusion medium consists of Krebs-Ringers bicarbonate-buffer (pH 7.4) containing 3% (w/v) bovine serum albumin (fraction V, Armour Inc.) and washed bovine red blood cells (HCT 25). Testes were perfused with this medium at a peristaltic perfusion flow of 10 ml/g hr at $34 \pm 0.5^\circ\text{C}$ in a constant temperature ($34.5 \pm 0.5^\circ\text{C}$) and humidified chamber unless noted otherwise. Glucose and NIH-LH-S₁₉, ovine were infused into the arterial cannula via a Sage micropump so that their final concentrations in the arterial perfusion medium were 1.0 mg/ml and 100 ng/ml, respectively. This concentration of LH assured maximal stimulation of all testes.

In the inhibitor experiments, one testis from an animal served as a control and the contralateral testis was infused with an inhibitor. Inhibitors (and steroids when indicated) were dissolved in 1 ml of ethanol (95%) and added at the designated concentration to 600 ml of perfusion media 12 hr before the perfusion to allow for equilibration. To 600 ml of control perfusion media was added 1 ml of 95% ethanol. Chubb and Ewing have given details (7).

In the structure-function experiments (see below) one testis from an animal was perfused *in vitro* as described above, and the contralateral testis was used for morphometric analysis.

Steroid Measurement

Testosterone was measured either with a high performance liquid chromatograph equipped with a flow-through UV spectrophotometer (8) or with a gas chromatograph (6). Testosterone biosynthetic intermediates were extracted from testicular venous effluent, separated and quantified by gas

chromatography as described by Chubb and Ewing (9).

Morphometric Analysis

Testes were perfusion fixed and embedded in Epon-Araldite. Sections were mounted on slides and stained. The volume density of Leydig cells (volume of Leydig cells per unit volume of testis) was obtained by counting points over Leydig cells and dividing by the total number of points counted over the testis. Leydig cell mass was determined according to Ewing et al. (10). Stereological analyses were used to determine the volume density of Leydig cell cytoplasmic organelles. Briefly, thin sections from each of the Epon-Araldite embedded blocks were mounted on 200-mesh Formvar grids and stained with uranyl acetate and lead citrate. The volume density of organelles was determined by counting points over a given organelle and dividing by the total number of points counted over Leydig cell cytoplasm. Details were described by Zirkin et al. (11).

Results and Discussion

In Vitro Perfused Testes Mimic *In Situ* Testicular Steroid Secretion

Evidence will be presented below which demonstrates that androgen biosynthesis and secretion by *in vitro* perfused rat and rabbit testes mimic *in situ* testicular steroidogenic function. Ewing and Eik-Nes (12) reported that *in vitro* perfused rabbit testes synthesized testosterone from acetate and cholesterol. They also demonstrated that LH stimulated testosterone secretion by *in vitro* perfused rabbit testes in a dose dependent fashion. More recent studies (6) revealed that adult rabbit testes perfused *in vitro* with a physiological concentration of LH secrete testosterone (T), dihydrotestosterone (DHT), 5α -androstan- $3\alpha,17\beta$ -diol ($3\alpha\text{DIOL}$) and 5α -androstan- $3\beta,17\beta$ -diol ($3\beta\text{DIOL}$) in amounts identical to that secreted *in situ* by rabbit testes.

Additional evidence that the *in vitro* perfused testis mimics *in situ* testicular steroidogenic function was derived from studies of testes from prepubertal, pubertal, post-pubertal and aged rabbits. Androgen secretion by testes from sexually maturing rabbits (Fig. 1) correlated closely with androgen concentration in peripheral blood (13). The steep increase in androgen secretion by *in vitro* perfused rabbit testes coincided with the onset of spermatogenesis and growth of the accessory sex organs (13). Of particular interest was the observa-

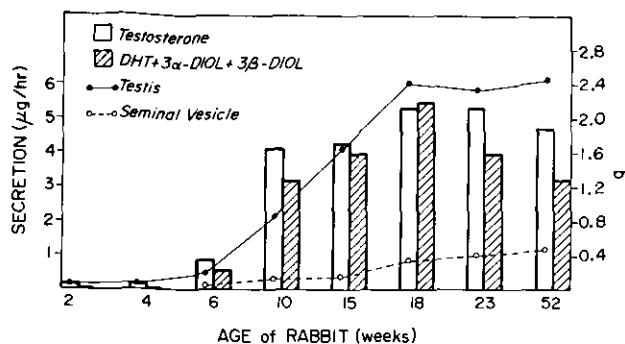


FIGURE 1. Secretion of testosterone and 5 α reduced androgens (DHT = dihydrotestosterone, 3 α DIOL = 5 α -androstan-3 α ,17 β -diol, 3 β DIOL = 5 α -androstan-3 β ,17 β -diol) by *in vitro* perfused testes from rabbits 2-52 weeks of age. Also shown are testis weight and seminal vesicle weight. Each bar or point represents six animals. Testes were perfused with artificial medium which contained 100 ng/ml of NIH-LH-S₁₉, ovine. Perfusions were carried out at 34.5°C, and the flow of perfusion medium through the testis-epididymis was maintained at a rate of 10 ml/g testis-hr.

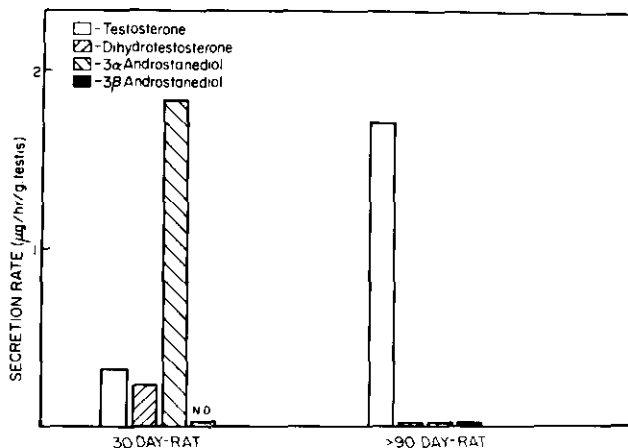


FIGURE 2. Secretion of testosterone, dihydrotestosterone, 3 α -androstanediol (5 α -androstan-3 α ,17 β -diol) and 3 β -androstanediol (5 α -androstan-3 β ,17 β -diol) by *in vitro* perfused testes from rats 30 days of age and greater than 90 days of age. Each bar represents the mean from five animals. Testes were perfused with artificial medium which contained 100 ng/ml of NIH-LH-S₁₉, ovine. Perfusions were carried out at 34.5°C, and the flow of perfusion medium through the testis-epididymis was maintained at a rate of 10 ml/g testis-hr.

tion that the ratio of T/DHT + 3 α DIOL + 3 β DIOL remained unchanged from 6-18 weeks in the sexually maturing rabbit (Fig. 1). This pattern of androgen production during sexual maturation in the rabbit is strikingly different than that reported for the rat during puberty (14-17). In contrast to the rabbit, the prepubertal rat testis produces 5 α reduced androgens but not testosterone. In the rat,

puberty is characterized by a progressive decline in the testicular synthesis of 5 α reduced androgens with a concomitant increase in testosterone production. We hypothesized that the prepubertal rat testis perfused *in vitro* would secrete 5 α reduced androgens rather than testosterone provided the *in vitro* perfused testis mimicked *in situ* testicular steroid production. The results in Figure 2 show that the 30 day-old prepubertal rat testis perfused *in vitro* secreted predominately 3 α DIOL. In contrast, the *in vitro* perfused adult rat testis secreted only testosterone. These results confirm that *in vitro* perfused rat and rabbit testes mimic the species pattern of androgen secretion in the prepubertal and postpubertal rat and rabbit. Finally, experiments have been completed demonstrating that testosterone secretion by *in vitro* perfused testes decreases with advanced age in rabbits concomitant with decreased spermatogenesis (18). Next we undertook a series of experiments designed to assess the effect of steroidogenic reaction inhibitors on testosterone secretion.

Acute Effects of Inhibitors of Steroidogenesis on Testosterone Biosynthesis and Secretion by *In Vitro* Perfused Testes

Fifteen reported steroidogenic reaction inhibitors were tested for their effects on testosterone secretion by rabbit testes perfused *in vitro* (Fig. 3). Testosterone secretion was inhibited from 1 to 95% by the chemicals tested. The results are expressed as the percentage inhibition (experimental T secretion \div control T secretion) \times 100. The compounds reported to inhibit the 17 α -hydroxylation reaction, SU-12054 (19), amphenone (20), spironolactone (19), and SKF-525-A (21), were among the most effective inhibitors at the 30 μ M concentration used. F 6060 (22), an inhibitor of the conversion of Δ^5 -3 β -hydroxy steroids to Δ^4 -3-ketosteroids and the cholesterol biosynthesis inhibitors AY-9944 (23) and triparanol (24) reduced testosterone secretion by 42%, 47%, and 24%, respectively. Inhibitors of testosterone 5 α reduction, gestonorone caproate (25), methotrexate (26), and 3-oxo-4-androsten-17 β -carboxylic acid (27), failed to inhibit testosterone secretion. Clomiphene citrate (28) and MK-665 (29) also inhibited testosterone secretion. SU-10603 (19), an inhibitor of the 17 α -hydroxylation and C-17,C-20 cleavage reactions, and medrogestone (30), an inhibitor of the conversion of Δ^5 -3 β -hydroxysteroids to Δ^4 -3-ketosteroids, decreased testosterone secretion by 95% and 92.5%, respectively. Aminoglu-

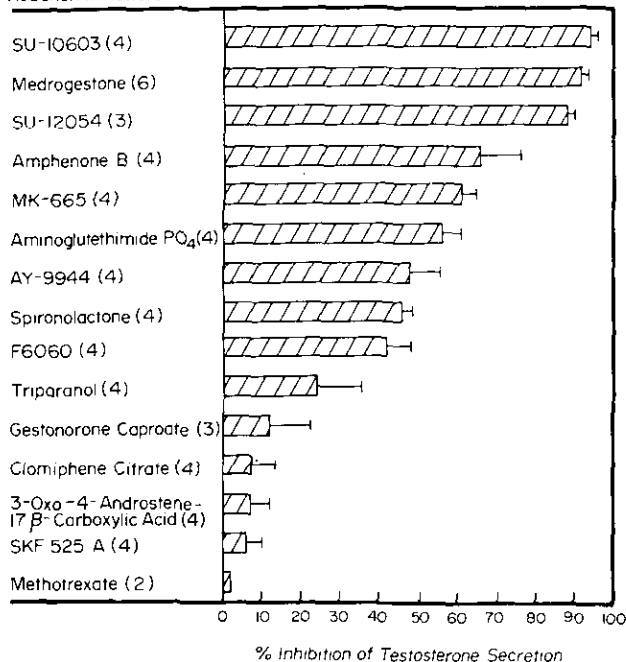


FIGURE 3. Effect of inhibitors on testosterone secretion by rabbit testes perfused *in vitro*. Inhibitors ($30 \mu M$) were added to the arterial perfusion media. Results are expressed as percentage of inhibition [$1 - (\text{experimental secretion rate} \div \text{control secretion rate}) \times 100$]. The T above each column denotes the SEM. The number of replicates is indicated in parentheses.

tethimide phosphate (31), an inhibitor of pregnenolone biosynthesis, reduced testosterone secretion by 55%.

SU-10603, medrogestone, and aminoglutethimide phosphate were selected for further study because they effectively inhibited testosterone secretion. Figure 4 illustrates the dose-response curves used to ascertain the minimum dose of each drug required to obtain maximum inhibition of testosterone secretion by rabbit testes. Testosterone secretion was inhibited completely by $40 \mu M$ medrogestone and SU-10603. Aminoglutethimide phosphate resulted in an 86% inhibition of testosterone secretion at $150 \mu M$ concentration. Similar results were forthcoming when rat testes were perfused *in vitro* in the presence of these three inhibitors (7).

The intratesticular testosterone content of rabbit testes perfused 4.5 hr with or without the inhibitors was measured to demonstrate that decreased testosterone secretion resulted from the inhibition of testosterone biosynthesis rather than its release. The low intratesticular testosterone content of the inhibited testes (Fig. 5) supported the argument that decreased testosterone secretion resulted from decreased testosterone biosynthesis. Taken togeth-

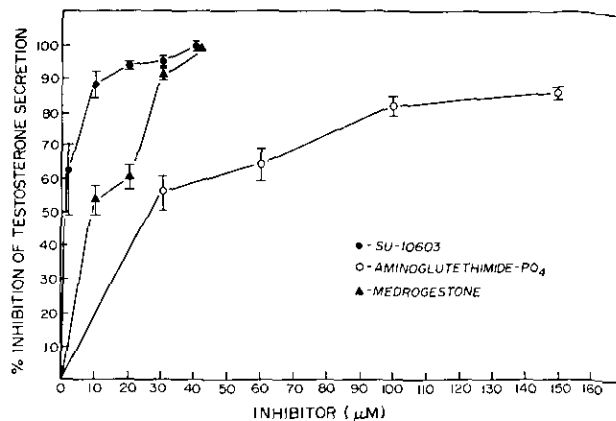


FIGURE 4. Dose-response curves for (●) SU-10603, (▲) medrogestone, and (○) aminoglutethimide phosphate. Testosterone secretion by control and experimental rabbit testes perfused *in vitro* was determined 2.5 hr after the perfusion was initiated. Results are expressed as percentage of inhibition [$1 - (\text{experimental secretion rate} \div \text{control secretion rate}) \times 100$]. Each point represents mean \pm SEM of six determinations. From Chubb and Ewing (7), by courtesy of the editor of American Journal of Physiology.

er, the results of these inhibitor studies suggest that the acute inhibition of steroidogenic enzymes is reflected by reduced testosterone secretion by *in vitro* perfused rat and rabbit testes.

Assessing the Site of Action of Inhibitors of Steroidogenesis in *In Vitro* Perfused Rat and Rabbit Testes

Diagnosis of defects in steroidogenesis associated with endocrine diseases has been based on the identification of the increase or decrease of individ-

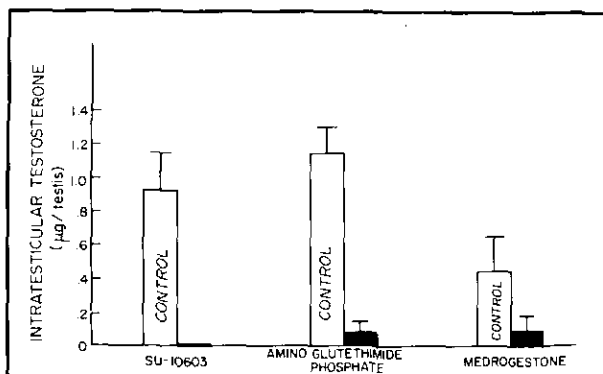


FIGURE 5. Effect of SU-10603 ($40 \mu M$), medrogestone ($40 \mu M$) and aminoglutethimide phosphate ($150 \mu M$) on testosterone content of rabbit testes perfused *in vitro* for 4.5 hr. Results are expressed as mean \pm SEM of six replicates.

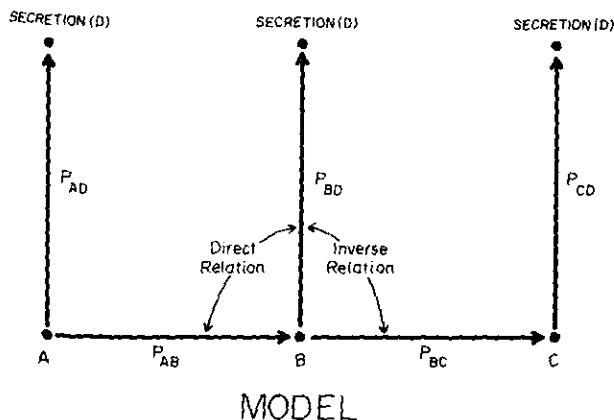


FIGURE 6. Model for measurement of steroidogenic reaction activity by steroid secretion rates. A, B, and C represent steroids that may be interconverted by one-way reactions (activities represented by P_{AB} and P_{BC}) or secreted (represented by P_{AD} , P_{BD} , and P_{CD}). From Chubb and Ewing (7), by courtesy of the editor of American Journal of Physiology.

ual steroid concentrations in urine, peripheral blood or the venous effluent of a steroidogenic organ (32-34). The model presented in Figure 6 illustrates this concept. A, B, and C represent steroids that are either interconverted by enzyme-catalyzed reactions (whose activities are represented by P_{AB} and P_{BC}) or secreted into the blood (P_{AD} , P_{BD} , and P_{CD}). Two assumptions are made for the model: (1) the tissue concentrations of A, B, and C are constant and (2) the conversions are one-way in the direction of the arrows. If the reaction converting B to C is inhibited, the secretion of B should increase (direct relation) whereas the secretion of C should decrease (inverse relation). Therefore, modification of the activity of a steroidogenic reaction in the *in vitro* perfused testis should be reflected by a change in the secretion of its substrate(s) and product(s).

Experimental evidence supporting this hypothesis was obtained by measuring the total endogenous steroid secretion rate of control and inhibitor-treated testes perfused *in vitro*. Figures 7-9 depict the effect of SU-10603, medrogestone, and aminoglutethimide phosphate on the secretion of testosterone and its biosynthetic intermediates by rat and rabbit testes perfused *in vitro*. The results are expressed as percentage of control secretion rate (experimental secretion rate/control secretion rate $\times 100$). The broken arrows in the figures denote the steroidogenic reaction(s) indicated to be inhibited. Three steroids formed from testosterone (DHT, 3α -DIOL, and 3β -DIOL) were also measured. However, these steroids always declined when testosterone secretion diminished and therefore were eliminated from the figures.

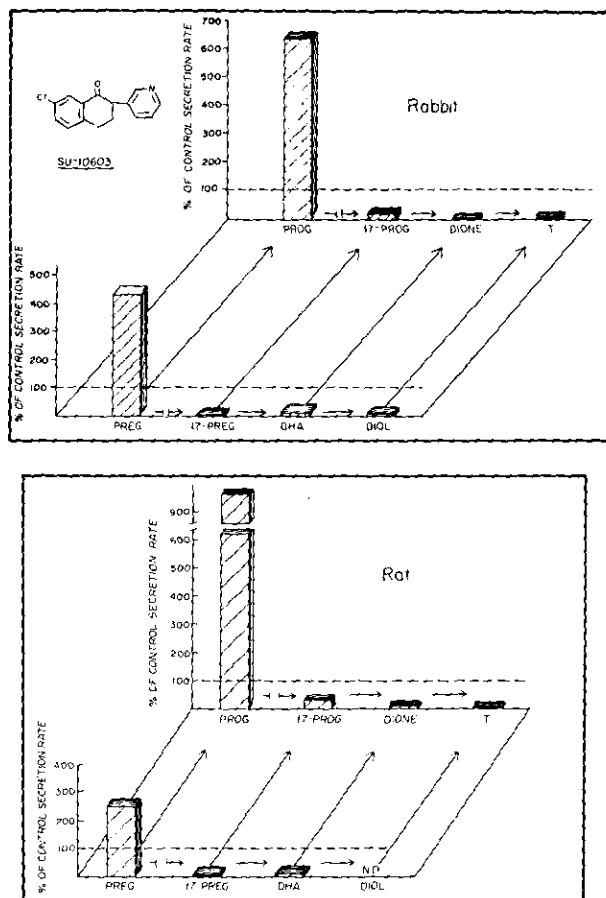


FIGURE 7. Effect of SU-10603 (40 μ M) on secretion of testosterone and its biosynthetic intermediates by rabbit and rat testes perfused *in vitro*. Testes were perfused for 4.5 hr and were maximally stimulated with LH. Results are expressed as percentage of control (experimental secretion rate \div control secretion rate $\times 100$). Dashed line represents 100% of control and broken arrow reflects the reaction indicated by results to be inhibited; $n = 6$ (rabbit) and $n = 4$ (rat); ND, non-detectable. From Chubb and Ewing (7), by courtesy of the editor of American Journal of Physiology.

SU-10603. SU-10603 inhibits the 17-hydroxylation reaction in rabbit and rat testes (Fig. 7). This site of action was indicated by the increased secretion of pregnenolone and progesterone, the reaction substrates, and the decreased secretion of the reaction products in both species. Subsequent experiments (7) demonstrated that SU-10603 also inhibited the C-17,C-20 cleavage reaction.

Medrogestone. Medrogestone inhibits the conversion of Δ^5 - 3β -hydroxysteroids to Δ^4 -3-ketosteroids in rabbit and rat testes (Fig. 8). This inhibition was demonstrated in rabbit testes by the secretion of the Δ^5 - 3β -hydroxysteroids (pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, and

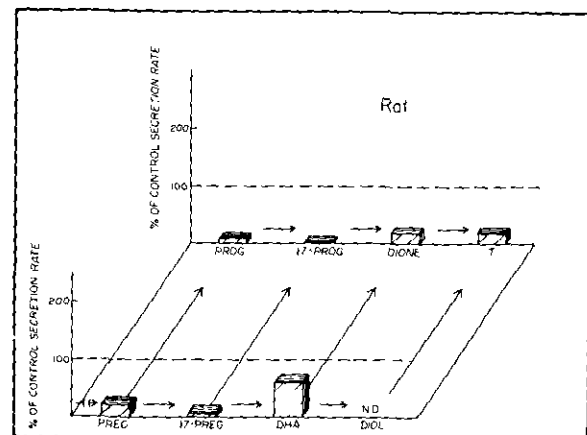
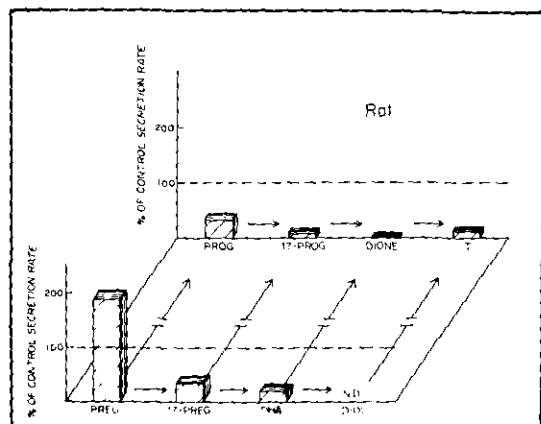
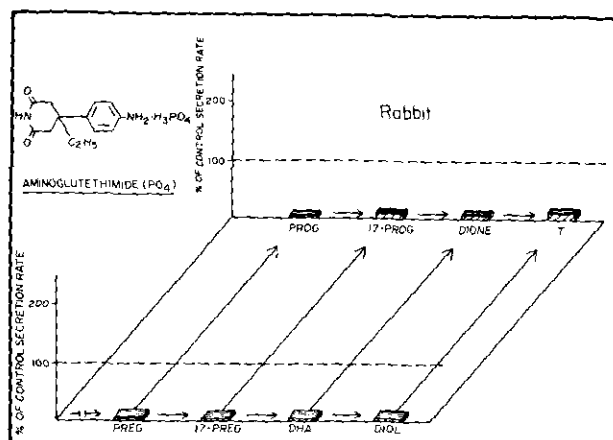
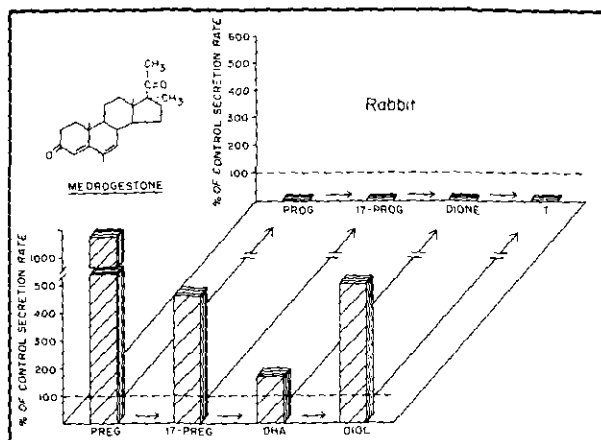


FIGURE 8. Effect of medrogestone $40 \mu\text{M}$ on secretion of testosterone and its biosynthetic intermediates by rabbit and rat testes perfused *in vitro*. Testes were perfused for 4.5 hr and were maximally stimulated with LH. Results are expressed as percentage of control (experimental secretion \div control secretion rate $\times 100$). Dashed line represents 100% of control and broken arrow reflects reaction indicated by results to be inhibited; $n = 6$ (rabbit) and $n = 4$ (rat); ND, nondetectable. From Chubb and Ewing (7), by courtesy of the editor of American Journal of Physiology.

FIGURE 9. Effect of aminoglutethimide phosphate ($150 \mu\text{M}$) on secretion of testosterone and its biosynthetic intermediates by rat and rabbit testes perfused *in vitro*. Testes were perfused for 4.5 hr and were maximally stimulated with LH. The results are expressed as percentage of control (experimental secretion rate \div control secretion rate $\times 100$). Dashed line represents 100% of control and broken arrow reflects reaction indicated by results to be inhibited; $n = 6$ (rabbit) and $n = 4$ (rat); ND, nondetectable. From Chubb and Ewing (7), by courtesy of the editor of American Journal of Physiology.

androstenediol) increasing above control levels in the rabbit and the secretion of the Δ^4 -3-ketosteroids (progesterone, 17-hydroxyprogesterone, androstenedione, and testosterone) decreasing to near zero in both rabbits and rat testes. Medrogestone also caused a partial inhibition of the 17-hydroxylation of pregnenolone in rat testes as indicated by the reduced secretion of 17-hydroxypregnenolone and dehydroepiandrosterone. Subsequently, it was shown that the simultaneous infusion of pregnenolone and medrogestone into *in vitro* perfused rat testes overcame this partial inhibition of 17-hydroxylation (7).

Aminoglutethimide Phosphate. Aminoglutethimide phosphate inhibits the secretion of testoster-

one and its Δ^5 -3 β -hydroxysteroid and Δ^4 -3-ketosteroid biosynthetic intermediates in perfused rabbit and rat testes (Fig. 9). An experiment to detect whether aminoglutethimide phosphate inhibits other testosterone biosynthetic reactions was performed by infusing pregnenolone ($3 \mu\text{g}/\text{ml}$) into aminoglutethimide phosphate inhibited rat and rabbit testes perfused *in vitro*. The resulting testosterone secretion rates were similar to those of control testes (7). These results suggest that aminoglutethimide phosphate inhibits only steroidogenic reactions forming pregnenolone.

Taken together, these data suggest that the activity of steroidogenic reactions in intact perfused

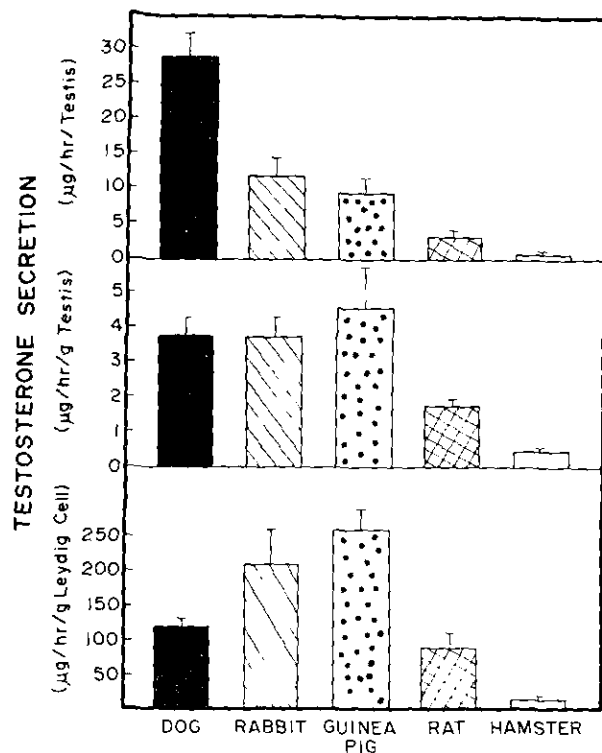


FIGURE 10. Testosterone secretion by dog, rabbit, guinea pig, rat and hamster testes perfused *in vitro* with artificial medium containing 100 ng/ml NIH LH S₁₉ (ovine): (upper panel) testosterone secretion per testis; (middle panel) testosterone secretion per g testis; (bottom panel) testosterone secretion per g Leydig cells. The T above each bar denotes the SEM ($n = 6$). From Ewing et al. (10), by courtesy of the editor of Endocrinology.

testes can be monitored by measuring the secretion of their substrates and product(s).

Correlation of Leydig Cell Structure with Steroidogenic Function

The steroidogenic enzymes responsible for converting cholesterol to testosterone are sequestered in mitochondrial and smooth endoplasmic reticulum

(SER) membranes of the Leydig cell (11). Efficient and coordinated function of these steroidogenic enzymes appear to depend on this membranous environment. Until recently, the quantitative relationship between mitochondrial and SER membranes and testicular steroidogenic function has not been studied. We discovered (10) that testosterone secretion varies dramatically between hamster, rat, dog, rabbit, and guinea pig testes *in vitro* under identical conditions (Fig. 10). The lower panel of Figure 10 demonstrates that significant between-species variation still was apparent when testosterone secretion was expressed on a $\mu\text{gT/hr-g}$ Leydig cell basis. We concluded that these differences in testosterone secretion could not be accounted for by differences in Leydig cell mass. An earlier publication (10) gives a more detailed discussion of the species differences in testosterone secretion.

We hypothesized that these species differences in testosterone secretion might be correlated with specific quantitative differences in the Leydig cell organelles sequestering steroidogenic enzymes. To test this hypothesis, stereological methods were employed to quantitate SER, mitochondria, rough endoplasmic reticulum (RER), and lipid in the Leydig cells of hamster, rat, rabbit, dog and guinea pig testes. The results obtained from ultrastructural analysis of one testis from each animal were compared to the testosterone secretion by the contralateral testis perfused *in vitro* with an artificial medium containing maximally stimulating concentrations of NIH-LH-S₁₉, ovine. Testes from the five species studied secreted testosterone at rates similar to those depicted earlier (Fig. 10). As expected from previous electron microscopic studies (1,2) Leydig cells from the five species demonstrated obvious differences in cytoplasmic organelles. A stereological analysis of Leydig cell ultrastructure (Table 1) confirmed these observations. Analysis of variance demonstrated significant ($p < 0.01$) between species differences in the volume densities of SER, mitochondria, RER, and lipid in the cytoplasm of Leydig cells (Table 1). Duncan's multiple range test demonstrated that SER volume density

Table 1. Volume densities of Leydig cell cytoplasmic organelles.^a

Animal	Volume density, % ^b			
	SER	Mitochondria	RER	Lipid
Dog	36.7 \pm 1.2	13.0 \pm 0.7	25.5 \pm 1.1	20.5 \pm 1.3
Rabbit	42.2 \pm 1.1	17.0 \pm 0.9	23.8 \pm 1.2	3.8 \pm 0.4
Guinea pig	60.9 \pm 0.9	18.2 \pm 0.7	8.9 \pm 0.4	7.9 \pm 0.7
Hamster	12.4 \pm 0.7	27.9 \pm 0.5	40.8 \pm 0.7	0.6 \pm 0.1
Rat	32.8 \pm 1.2	19.7 \pm 0.7	30.1 \pm 1.0	0.3 \pm 0.1

^a Data from Zirkin et al. (11).

^b Volume densities expressed as % Leydig cell cytoplasmic volume, mean \pm SEM.

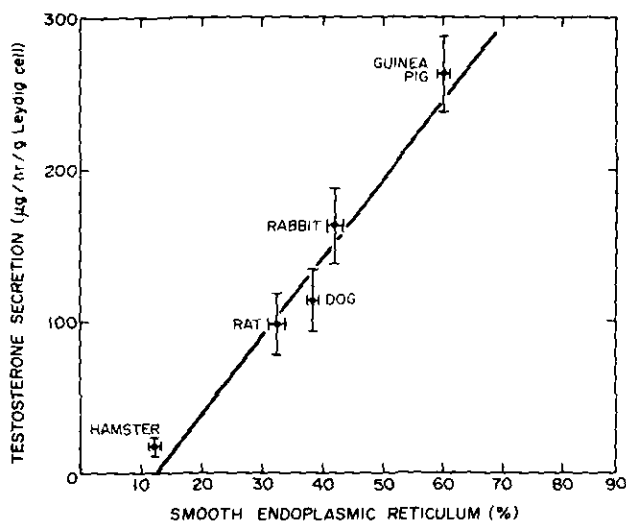


FIGURE 11. Plot of mean testosterone secretion by maximally stimulated *in vitro* perfused testes of hamster, rat, dog, rabbit, and guinea pig ($n = 5$ for each species) against mean volume density of SER in Leydig cell cytoplasm of contralateral testes. A linear, positive correlation ($r = 0.99$) is seen. From Zirkin et al. (11), by courtesy of the editor of *Endocrinology*.

(proportion of Leydig cell cytoplasm occupied by SER) was significantly higher ($p < 0.05$) in the guinea pig and lower ($p < 0.05$) in the hamster than in the other species; rabbit and dog SER volume densities were similar and both significantly higher ($p < 0.05$) than rat. RER volume densities essentially were the inverse of SER. In the case of mitochondria (Table 1), volume density was significantly higher ($p < 0.05$) in hamster and lower ($p < 0.05$) in dog than in the other species and intermediate and similar in rabbit, guinea pig and rat. Lipid was relatively rare in four of the five species, with dog being the exception.

Regression analysis (Fig. 11) demonstrated that a linear and positive correlation ($r = 0.99$) existed between testosterone secretion, expressed as $\mu\text{g T/hr-g Leydig cell}$, and the mean volume density of SER in Leydig cell cytoplasm. This correlation was highly significant ($p < 0.005$). Significant positive correlations were not found between testosterone secretion and the volume density of any other Leydig cell organelle. Interestingly, testosterone secretion was significantly ($p < 0.005$) but negatively correlated ($r = -0.99$) with the volume density of RER.

These results demonstrate for the first time, that virtually all the differences in testosterone secretion by *in vitro* perfused, maximally gonadotropin stimulated testes of five laboratory animals can be accounted for by the proportion of Leydig cell cytoplasm occupied by SER. This implies a close

correlation between Leydig cell ultrastructure and steroidogenesis.

Conclusion

Androgen secretion by *in vitro* perfused rat and rabbit testes mimics testicular steroidogenic function *in situ*. Inhibitions of Leydig cell steroidogenic enzymes is reflected by reduced testosterone biosynthesis and secretion by *in vitro* perfused rat and rabbit testes. Moreover, the activity of specific steroidogenic reactions can be monitored by measuring the secretion of reaction substrate(s) and product(s) from *in vitro* perfused testes. Testosterone secretion by *in vitro* perfused testes from five species is highly and positively correlated with the volume density of smooth endoplasmic reticulum in Leydig cell cytoplasm.

Exploitation of these findings will allow toxicologists to quantitatively assess the effect of toxicants on Leydig cell testosterone biosynthesis and secretion, to identify the specific steroidogenic enzymes affected, to assess whether the membranous environment of the steroidogenic enzymes is compromised, and perhaps even to predict the deleterious effect of a toxic agent on Leydig cell steroidogenic function from a stereological assessment of Leydig cell ultrastructure.

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